Inducible neuronal PrP knockout mice reveal potential therapeutic window for TSE intervention

Inducible Neuronal PrP Knockout Mice Reveal Potential Therapeutic Window for TSE Intervention.

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Introduction

The aim of this project is to investigate the effect of removing prion protein (PrP) expression from neurones of the central nervous system and to determine the subsequent response to infection with the transmissible spongiform encephalopathy (TSE) agent. We have produced and tested a model in which the PrP gene is conditionally deleted in neurones by a tamoxifen-activated Cre. Removal of neuronal PrP prior to infection revealed greatly extended incubation periods and altered pathology. In the study presented here, the knockout of neuronal PrP was performed in groups of mice at various specified times post-infection to determine the effect on disease progression.

Inducible neuronal PrP knockout model

Inducible neuronal PrP knockout mice display reduced total PrP expression in brain after tamoxifen induction (figure 1a and 1b), with extended incubation period (figure 1c) and altered terminal neuropathology (figure 2) following subsequent ME7 infection.

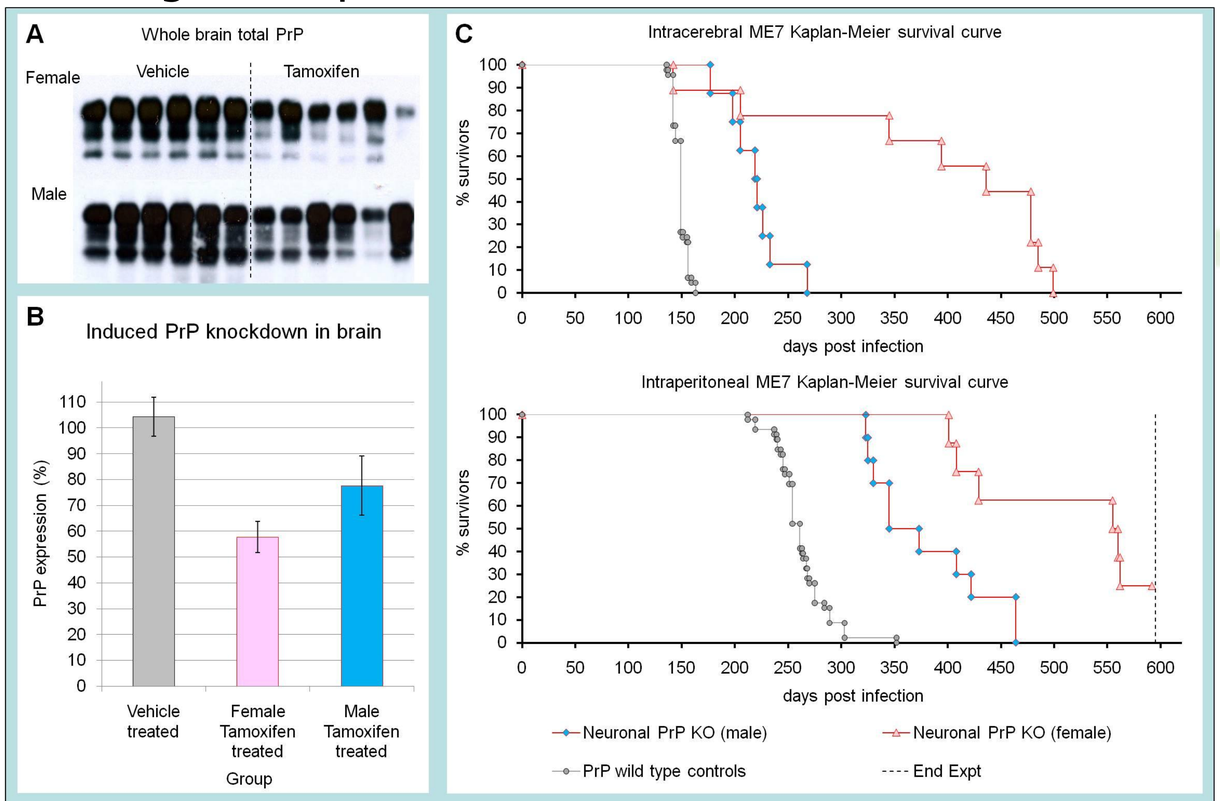

Figure 1. A, Western blot analysis of whole brain homogenate following vehicle or tamoxifen treatment. PrP visualised with antibody 7A12. **B,** Densitometric analysis of whole brain PrP shown as group mean PrP expression +/- SEM. **C,** Survival curve post ME7 challenge, showing extended incubation periods in neuronal PrP knockout mice (red lines), and differential response by sex, compared to mice expressing PrP normally.

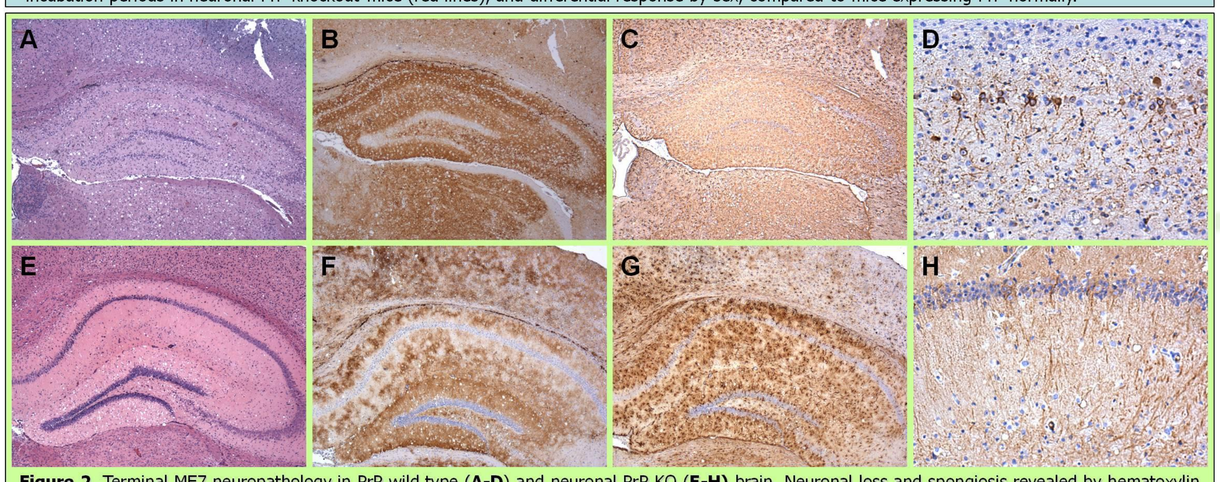

Figure 2. Terminal ME7 neuropathology in PrP wild type (**A-D**) and neuronal PrP KO (**E-H**) brain. Neuronal loss and spongiosis revealed by hematoxylin and eosin stain (**A,E**); differences in PrP deposition (**B,F**); GFAP (**C,G**); and MAP2 (**D,H**); revealed by immunohistohemistry with relevant antibodies.

Timed intervention during disease using the inducible neuronal PrP knockout model

Induction of PrP knockout in neurones of male mice at various time-points during ME7 infection (figure 3) show that the progression of disease can be altered prior to, but not during the clinical phase of disease (figure 4). These findings suggest that a potential window of opportunity for therapeutic intervention involving PrP depletion exists during TSE disease. However, once disease has progressed to the point where overt clinical signs are recognised, the depletion of neuronal PrP then has minimal impact upon the final incubation period.

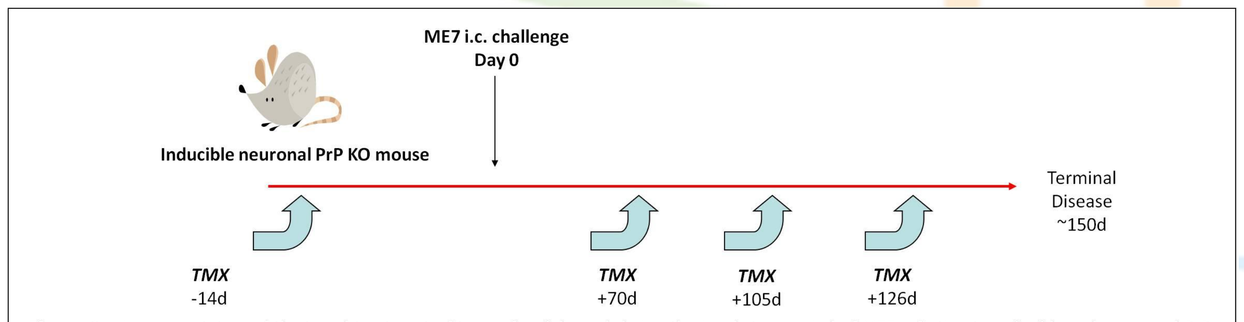

Figure 3. A representation of the timed treatment of tamoxifen delivered during the incubation period of ME7 infection to inducible male neuronal PrP knockout mice (n=5) . The 126day delivery time-point is during the clinical phase of disease.

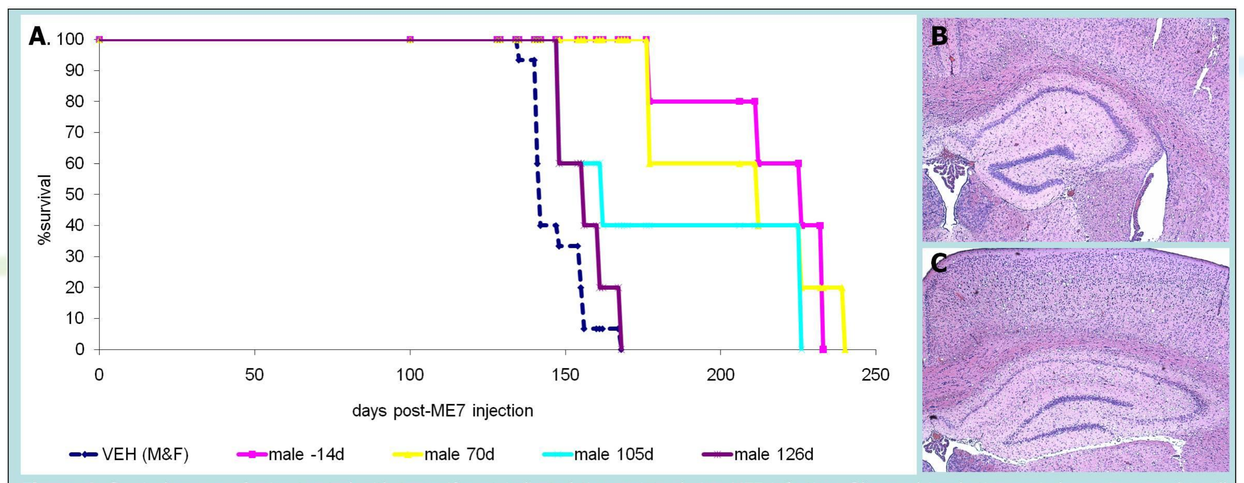

Figure 4. Survival curves of mice treated with tamoxifen at indicated time-points during ME7 infection. Observed incubation periods are increased in all neuronal PrP knockout groups except the 126d treatment time-point, which occurs during the clinical phase of disease **(A)**. Representative H&E sections of the hippocampal regions of male mice treated at either 14d prior **(B)** or 126d post **(C)** injection with ME7. Further pathological analysis is underway in this model to establish whether further changes in pathology are observed following neuronal PrP knockout at different time-points.

Summary

Knockout of PrP from central nervous system neurones during disease prolongs incubation times at all time-points examined except during the clinical phase of disease – these findings may have implications for timing therapeutic interventions in TSE disease.

The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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